

## WHAT IS CLAIMED IS:

1. An isolated or recombinant nucleic acid comprising  
a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:5  
over a region of at least about 100 residues,  
5 a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:7  
over a region of at least about 100 residues,  
a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:11  
over a region of at least about 100 residues,  
a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:13  
10 over a region of at least about 100 residues, or  
a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:15  
over a region of at least about 100 residues,  
wherein the nucleic acid encodes at least one polypeptide having an amylase  
activity, and the sequence identities are determined by analysis with a sequence  
15 comparison algorithm or by a visual inspection.
2. The isolated or recombinant nucleic acid of claim 1, wherein the  
nucleic acid comprises  
a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:5  
20 over a region of at least about 200 residues,  
a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:7  
over a region of at least about 200 residues,  
a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:11  
over a region of at least about 200 residues,  
25 a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:13  
over a region of at least about 200 residues, or  
a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:15  
over a region of at least about 200 residues.
3. The isolated or recombinant nucleic acid of claim 2, wherein the  
nucleic acid comprises  
a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:5  
over a region of at least about 300 residues,

a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:7 over a region of at least about 300 residues,

a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:11 over a region of at least about 300 residues,

5 a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:13 over a region of at least about 300 residues,

a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:15 over a region of at least about 300 residues.

10 4. The isolated or recombinant nucleic acid of claim 3, wherein the nucleic acid comprises

a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:5 over a region of at least about 400 residues,

15 a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:7 over a region of at least about 400 residues,

a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:11 over a region of at least about 400 residues,

a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:13 over a region of at least about 400 residues, or

20 a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:15 over a region of at least about 400 residues.

5. The isolated or recombinant nucleic acid of claim 4, wherein the nucleic acid comprises

25 a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:5 over a region of at least about 500 residues,

a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:7 over a region of at least about 500 residues,

30 a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:11 over a region of at least about 500 residues,

a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:13 over a region of at least about 500 residues, or

a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:15 over a region of at least about 500 residues.

6. The isolated or recombinant nucleic acid of claim 5, wherein the nucleic acid comprises

a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:5  
over a region of at least about 600 residues,

a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:7  
over a region of at least about 600 residues,

a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:11  
over a region of at least about 600 residues,

a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:13  
over a region of at least about 600 residues, or

a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:15  
over a region of at least about 600 residues.

7. The isolated or recombinant nucleic acid of claim 6, wherein the nucleic acid comprises

a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:5  
over a region of at least about 700 residues,

a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:7  
over a region of at least about 700 residues,

a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:11  
over a region of at least about 700 residues,

a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:13  
over a region of at least about 700 residues, or

a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:15  
over a region of at least about 700 residues.

8. The isolated or recombinant nucleic acid of claim 7, wherein the nucleic acid comprises

a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:5  
over a region of at least about 800 residues,

a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:7  
over a region of at least about 800 residues,

a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:11  
over a region of at least about 800 residues,

a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:13  
over a region of at least about 800 residues, or

5 a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:15  
over a region of at least about 800 residues.

9. The isolated or recombinant nucleic acid of claim 8, wherein the  
nucleic acid comprises

10 a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:5  
over a region of at least about 900 residues,

a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:7  
over a region of at least about 900 residues,

15 a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:11  
over a region of at least about 900 residues,

a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:13  
over a region of at least about 900 residues, or

a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:15  
over a region of at least about 900 residues.

20 10. The isolated or recombinant nucleic acid of claim 9, wherein the  
nucleic acid comprises

a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:5  
over a region of at least about 1000 residues,

25 a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:7  
over a region of at least about 1000 residues,

a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:11  
over a region of at least about 1000 residues,

30 a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:13  
over a region of at least about 1000 residues, or

a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:15  
over a region of at least about 1000 residues.

11. The isolated or recombinant nucleic acid of claim 1, wherein the nucleic acid comprises

a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:5 over a region of at least about 100 residues,

5 a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:7 over a region of at least about 100 residues,

a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:11 over a region of at least about 100 residues,

a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:13 over a region of at least about 100 residues, or

a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:15 over a region of at least about 100 residues.

12. The isolated or recombinant nucleic acid of claim 11, wherein the nucleic acid comprises

a nucleic acid sequence having at least 98% sequence identity to SEQ ID NO:5 over a region of at least about 100 residues,

a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:7 over a region of at least about 100 residues,

20 a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:11 over a region of at least about 100 residues,

a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:13 over a region of at least about 100 residues, or

25 a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:15 over a region of at least about 100 residues.

13. The isolated or recombinant nucleic acid of claim 12, wherein the nucleic acid sequence comprises

30 a nucleic acid sequence having at least 99% sequence identity to SEQ ID NO:5 over a region of at least about 100 residues,

a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:7 over a region of at least about 100 residues,

a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:11 over a region of at least about 100 residues,

a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:13  
over a region of at least about 100 residues, or

a nucleic acid sequence having at least 98% sequence identity to SEQ ID NO:15  
over a region of at least about 100 residues.

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14. The isolated or recombinant nucleic acid of claim 13, wherein the  
nucleic acid comprises

a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:7  
over a region of at least about 100 residues,

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a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:11  
over a region of at least about 100 residues,

a nucleic acid sequence having at least 98% sequence identity to SEQ ID NO:13  
over a region of at least about 100 residues, or

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a nucleic acid sequence having at least 99% sequence identity to SEQ ID NO:15  
over a region of at least about 100 residues.

15. The isolated or recombinant nucleic acid of claim 14, wherein the  
nucleic acid sequence comprises

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a nucleic acid sequence having at least 98% sequence identity to SEQ ID NO:7  
over a region of at least about 100 residues,

a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:11  
over a region of at least about 100 residues, or

a nucleic acid sequence having at least 99% sequence identity to SEQ ID NO:13  
over a region of at least about 100 residues.

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16. The isolated or recombinant nucleic acid of claim 15, wherein the  
nucleic acid comprises

a nucleic acid sequence having at least 99% sequence identity to SEQ ID NO:7  
over a region of at least about 100 residues,

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a nucleic acid sequence having at least 98% sequence identity to SEQ ID NO:11  
over a region of at least about 100 residues.

17. The isolated or recombinant nucleic acid of claim 16, wherein the  
nucleic acid comprises

a nucleic acid sequence having at least 99% sequence identity to SEQ ID NO:11 over a region of at least about 100 residues.

18. The isolated or recombinant nucleic acid of claim 1, wherein the nucleic acid sequence comprises

a sequence as set forth in SEQ ID NO:1,  
a sequence as set forth in SEQ ID NO:5,  
a sequence as set forth in SEQ ID NO:7,  
a sequence as set forth in SEQ ID NO:11,  
a sequence as set forth in SEQ ID NO:13, or  
a sequence as set forth in SEQ ID NO:15.

19. The isolated or recombinant nucleic acid of claim 1, wherein the nucleic acid sequence encodes a polypeptide comprising

a polypeptide having a sequence as set forth in SEQ ID NO:2,  
a polypeptide having a sequence as set forth in SEQ ID NO:6,  
a polypeptide having a sequence as set forth in SEQ ID NO:8,  
a polypeptide having a sequence as set forth in SEQ ID NO:12,  
a polypeptide having a sequence as set forth in SEQ ID NO:14, or  
a polypeptide having a sequence as set forth in SEQ ID NO:16.

20. The isolated or recombinant nucleic acid of claim 1, wherein the sequence comparison algorithm is a BLAST version 2.2.2 algorithm where a filtering setting is set to blastall -p blastp -d "nr pataa" -F F, and all other options are set to default.

21. The isolated or recombinant nucleic acid of claim 1, wherein the amylase activity comprises hydrolyzing glucosidic bonds.

22. The isolated or recombinant nucleic acid of claim 21, wherein the amylase activity comprises a glucoamylase activity.

23. The isolated or recombinant nucleic acid of claim 21, wherein the amylase activity comprises a 1,4- $\alpha$ -D-glucan glucohydrolase activity.

24. The isolated or recombinant nucleic acid of claim 21, wherein the amylase activity comprises an  $\alpha$ -amylase activity.

25. The isolated or recombinant nucleic acid of claim 21, wherein the  
5 amylase activity comprises an exoamylase activity.

26. The isolated or recombinant nucleic acid of claim 21, wherein the amylase activity comprises a  $\beta$ -amylase activity.

10 27. The isolated or recombinant nucleic acid of claim 21, wherein the glucosidic bonds comprise an  $\alpha$ -1,4-glucosidic bond.

28. The isolated or recombinant nucleic acid of claim 21, wherein the glucosidic bonds comprise an  $\alpha$ -1,6-glucosidic bond.

15 29. The isolated or recombinant nucleic acid of claim 21, wherein the amylase activity comprises hydrolyzing glucosidic bonds in a starch.

20 30. The isolated or recombinant nucleic acid of claim 29, wherein the amylase activity further comprises hydrolyzing glucosidic bonds in the starch to produce maltodextrines.

25 31. The isolated or recombinant nucleic acid of claim 21, wherein the amylase activity comprises cleaving a maltose or a D-glucose unit from non-reducing end of the starch.

32. The isolated or recombinant nucleic acid of claim 1, wherein the amylase activity is thermostable.

30 33. The isolated or recombinant nucleic acid of claim 32, wherein the polypeptide retains an amylase activity under conditions comprising a temperature range of between about 37°C to about 95°C.



34. The isolated or recombinant nucleic acid of claim 33, wherein the polypeptide retains an amylase activity under conditions comprising a temperature range of between about 55°C to about 85°C.

5 35. The isolated or recombinant nucleic acid of claim 33, wherein the polypeptide retains an amylase activity under conditions comprising a temperature range of between about 70°C to about 95°C.

10 36. The isolated or recombinant nucleic acid of claim 35, wherein the polypeptide retains an amylase activity under conditions comprising a temperature range of between about 90°C to about 95°C.

15 37. The isolated or recombinant nucleic acid of claim 1, wherein the amylase activity is thermotolerant.

38. The isolated or recombinant nucleic acid of claim 37, wherein the polypeptide retains an amylase activity after exposure to a temperature in the range from greater than 37°C to about 95°C.

20 39. The isolated or recombinant nucleic acid of claim 38, wherein the polypeptide retains an amylase activity after exposure to a temperature in the range from greater than 55°C to about 85°C.

25 40. The isolated or recombinant nucleic acid of claim 38, wherein the polypeptide retains an amylase activity after exposure to a temperature in the range from greater than 90°C to about 95°C.

30 41. An isolated or recombinant nucleic acid, wherein the nucleic acid comprises a sequence that hybridizes under stringent conditions to a nucleic acid comprising

a sequence as set forth in SEQ ID NO:1,  
a sequence as set forth in SEQ ID NO:5,  
a sequence as set forth in SEQ ID NO:7,  
a sequence as set forth in SEQ ID NO:11,

a sequence as set forth in SEQ ID NO:13, or  
a sequence as set forth in SEQ ID NO:15,  
wherein the nucleic acid encodes a polypeptide having an amylase activity.

5                   42.    The isolated or recombinant nucleic acid of claim 41, wherein the  
nucleic acid is at least about 100 residues in length.

                  43.    The isolated or recombinant nucleic acid of claim 42, wherein the  
nucleic acid is at least about 200, 300, 400 residues in length.

10                   44.    The isolated or recombinant nucleic acid of claim 43, wherein the  
nucleic acid is at least about 500, 600, 700, 800, 900, 1000 residues in length or the full  
length of the gene or transcript.

15                   45.    The isolated or recombinant nucleic acid of claim 41, wherein the  
stringent conditions include a wash step comprising a wash in 0.2X SSC at a temperature  
of about 65°C for about 15 minutes.

20                   46.    A nucleic acid probe for identifying a nucleic acid encoding a  
polypeptide with an amylase activity, wherein the probe comprises at least 10 consecutive  
bases of a sequence comprising:

                  a sequence as set forth in SEQ ID NO:1,  
                  a sequence as set forth in SEQ ID NO:5,  
                  a sequence as set forth in SEQ ID NO:7,  
25                   a sequence as set forth in SEQ ID NO:11,  
                  a sequence as set forth in SEQ ID NO:13, or  
                  a sequence as set forth in SEQ ID NO:15,

wherein the probe identifies the nucleic acid by binding or hybridization.

30                   47.    The nucleic acid probe of claim 46, wherein the probe comprises an  
oligonucleotide comprising at least about 10 to 50, about 20 to 60, about 30 to 70, about 40  
to 80, or about 60 to 100 consecutive bases of a sequence comprising

                  a sequence as set forth in SEQ ID NO:1,  
                  a sequence as set forth in SEQ ID NO:5,

a sequence as set forth in SEQ ID NO:7,  
a sequence as set forth in SEQ ID NO:11,  
a sequence as set forth in SEQ ID NO:13, or  
a sequence as set forth in SEQ ID NO:15.

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48. A nucleic acid probe for identifying a nucleic acid encoding a peptide having an amylase activity, wherein the probe comprises a nucleic acid comprising

a sequence as set forth in SEQ ID NO:1,  
a nucleic acid sequence having at least 95% sequence identity to SEQ ID NO:1 over a region of at least about 100 residues,  
a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:7 over a region of at least about 100 residues,  
a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:11 over a region of at least about 100 residues,  
a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:13 over a region of at least about 100 residues, or  
a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:15 over a region of at least about 100 residues,  
wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection.

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49. The nucleic acid probe of claim 48, wherein the probe comprises an oligonucleotide comprising at least about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, or about 60 to 100 consecutive bases of a nucleic acid sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:5, or a subsequence thereof, a sequence as set forth in SEQ ID NO:7, or a subsequence thereof, a sequence as set forth in SEQ ID NO:11, or a subsequence thereof, a sequence as set forth in SEQ ID NO:13, or a subsequence thereof, a sequence as set forth in SEQ ID NO:15, or a subsequence thereof.

50. The nucleic acid probe of claim 48, wherein the probe comprises a nucleic acid sequence having at least 90% sequence identity to a region of at least about 100 residues of a nucleic acid comprising a sequence as set forth in SEQ ID NO:5, or a

subsequence thereof, a sequence as set forth in SEQ ID NO:7, or a subsequence thereof, a sequence as set forth in SEQ ID NO:11, or a subsequence thereof, a sequence as set forth in SEQ ID NO:13, or a subsequence thereof, a sequence as set forth in SEQ ID NO:15, or a subsequence thereof.

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51. The nucleic acid probe of claim 50, wherein the probe comprises a nucleic acid sequence having at least 95% sequence identity to a region of at least about 100 residues of a nucleic acid comprising a sequence as set forth in SEQ ID NO:5, or a subsequence thereof, a sequence as set forth in SEQ ID NO:7, or a subsequence thereof, a sequence as set forth in SEQ ID NO:11, or a subsequence thereof, a sequence as set forth in SEQ ID NO:13, or a subsequence thereof, a sequence as set forth in SEQ ID NO:15, or a subsequence thereof.

52. The nucleic acid probe of claim 51, wherein the probe comprises a nucleic acid sequence having at least 98% sequence identity to a region of at least about 100 residues of a nucleic acid comprising a sequence as set forth in SEQ ID NO:5, or a subsequence thereof, a sequence as set forth in SEQ ID NO:7, or a subsequence thereof, a sequence as set forth in SEQ ID NO:11, or a subsequence thereof, a sequence as set forth in SEQ ID NO:13, or a subsequence thereof, a sequence as set forth in SEQ ID NO:15, or a subsequence thereof.

53. An amplification primer sequence pair for amplifying a nucleic acid encoding a polypeptide having an amylase activity, wherein the primer pair is capable of amplifying a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, SEQ ID NO:5, or a subsequence thereof, a sequence as set forth in SEQ ID NO:7, or a subsequence thereof, a sequence as set forth in SEQ ID NO:11, or a subsequence thereof, a sequence as set forth in SEQ ID NO:13, or a subsequence thereof, a sequence as set forth in SEQ ID NO:15, or a subsequence thereof.

54. The amplification primer pair of claim 53, wherein each member of the amplification primer sequence pair comprises an oligonucleotide comprising at least about 10 to 50 consecutive bases of the sequence.

55. A method of amplifying a nucleic acid encoding a polypeptide having an amylase activity comprising amplification of a template nucleic acid with an amplification primer sequence pair capable of amplifying a nucleic acid sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:5, or a subsequence thereof, a sequence as set forth in SEQ ID NO:7, or a subsequence thereof, a sequence as set forth in SEQ ID NO:11, or a subsequence thereof, a sequence as set forth in SEQ ID NO:13, or a subsequence thereof, a sequence as set forth in SEQ ID NO:15, or a subsequence thereof.

56. An expression cassette comprising a nucleic acid comprising:  
(i) a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:5 over a region of at least about 100 residues,  
a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:7 over a region of at least about 100 residues,  
a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:11 over a region of at least about 100 residues,  
a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:13 over a region of at least about 100 residues, or  
a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:15 over a region of at least about 100 residues,  
wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection; or,  
(ii) a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:5, or a subsequence thereof, a sequence as set forth in SEQ ID NO:7, or a subsequence thereof, a sequence as set forth in SEQ ID NO:11, or a subsequence thereof, a sequence as set forth in SEQ ID NO:13, or a subsequence thereof, a sequence as set forth in SEQ ID NO:15, or a subsequence thereof.

57. A vector comprising a nucleic acid comprising  
(i) a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:5 over a region of at least about 100 residues,  
a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:7 over a region of at least about 100 residues,

a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:11 over a region of at least about 100 residues,

a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:13 over a region of at least about 100 residues, or

5 a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:15 over a region of at least about 100 residues,

wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection; or,

(ii) a nucleic acid that hybridizes under stringent conditions to a nucleic acid  
10 comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:5, or a subsequence thereof, a sequence as set forth in SEQ ID NO:7, or a subsequence thereof, a sequence as set forth in SEQ ID NO:11, or a subsequence thereof, a sequence as set forth in SEQ ID NO:13, or a subsequence thereof, a sequence as set forth in SEQ ID NO:15, or a subsequence thereof.

15 58. A cloning vehicle comprising a vector as set forth in claim 57, wherein the cloning vehicle comprises a viral vector, a plasmid, a phage, a phagemid, a cosmid, a fosmid, a bacteriophage or an artificial chromosome.

20 59. The cloning vehicle of claim 58, wherein the viral vector comprises an adenovirus vector, a retroviral vector or an adeno-associated viral vector.

25 60. The cloning vehicle of claim 53, comprising a bacterial artificial chromosome (BAC), a plasmid, a bacteriophage P1-derived vector (PAC), a yeast artificial chromosome (YAC), or a mammalian artificial chromosome (MAC).

61. A transformed cell comprising a vector, wherein the vector comprises

(i) a nucleic acid sequence having at least 90% sequence identity to SEQ ID  
30 NO:5 over a region of at least about 100 residues,

a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:7 over a region of at least about 100 residues,

a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:11 over a region of at least about 100 residues,

a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:13 over a region of at least about 100 residues, or

a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:15 over a region of at least about 100 residues,

5 wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection; or,

(ii) a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:5, or a subsequence thereof, a sequence as set forth in SEQ ID NO:7, or a subsequence thereof, a sequence as set forth in SEQ ID NO:11, or a subsequence thereof, a sequence as set forth in SEQ ID NO:13, or a subsequence thereof, a sequence as set forth in SEQ ID NO:15, or a subsequence thereof.

62. A transformed cell comprising

15 (i) a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:5 over a region of at least about 100 residues,

a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:7 over a region of at least about 100 residues,

20 a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:11 over a region of at least about 100 residues,

a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:13 over a region of at least about 100 residues, or

a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:15 over a region of at least about 100 residues,

25 wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection; or,

(ii) a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:5, or a subsequence thereof, a sequence as set forth in SEQ ID NO:7, or a subsequence thereof, a sequence as set forth in SEQ ID NO:11, or a subsequence thereof, a sequence as set forth in SEQ ID NO:13, or a subsequence thereof, a sequence as set forth in SEQ ID NO:15, or a subsequence thereof.

63. The transformed cell of claim 61 or claim 62, wherein the cell is a bacterial cell, a mammalian cell, a fungal cell, a yeast cell, an insect cell or a plant cell.

64. A transgenic non-human animal comprising

5 (i) a nucleic acid sequence as set forth in SEQ ID NO:1,  
a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:5 over a region of at least about 100 residues,  
a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:7 over a region of at least about 100 residues,

10 a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:11 over a region of at least about 100 residues,  
a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:13 over a region of at least about 100 residues, or  
a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:15 over a region of at least about 100 residues,

15 wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection; or,

(ii) a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:5, or a subsequence thereof, a sequence as set forth in SEQ ID

20 NO:7, or a subsequence thereof, a sequence as set forth in SEQ ID NO:11, or a subsequence thereof, a sequence as set forth in SEQ ID NO:13, or a subsequence thereof, a sequence as set forth in SEQ ID NO:15, or a subsequence thereof.

25 65. The transgenic non-human animal of claim 64, wherein the animal is a mouse.

66. A transgenic plant comprising

(i) a nucleic acid sequence as set forth in SEQ ID NO:1,  
30 a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:5 over a region of at least about 100 residues,  
a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:7 over a region of at least about 100 residues,



a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:11 over a region of at least about 100 residues,

a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:13 over a region of at least about 100 residues, or

5 a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:15 over a region of at least about 100 residues,

wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection; or,

(ii) a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:5, or a subsequence thereof, a sequence as set forth in SEQ ID NO:7, or a subsequence thereof, a sequence as set forth in SEQ ID NO:11, or a subsequence thereof, a sequence as set forth in SEQ ID NO:13, or a subsequence thereof, a sequence as set forth in SEQ ID NO:15, or a subsequence thereof.

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67. The transgenic plant of claim 66, wherein the plant is a corn plant, a sorghum plant, a potato plant, a tomato plant, a wheat plant, an oilseed plant, a rapeseed plant, a soybean plant, a rice plant, a barley plant, a grass, or a tobacco plant.

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68. A transgenic seed comprising

(i) a nucleic acid sequence as set forth in SEQ ID NO:1,

a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:5 over a region of at least about 100 residues,

a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:7 over a region of at least about 100 residues,

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a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:11 over a region of at least about 100 residues,

a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:13 over a region of at least about 100 residues, or

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a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:15 over a region of at least about 100 residues,

wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection; or,

(ii) a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:5, or a subsequence thereof, a sequence as set forth in SEQ ID NO:7, or a subsequence thereof, a sequence as set forth in SEQ ID NO:11, or a subsequence thereof, a sequence as set forth in SEQ ID NO:13, or a subsequence thereof, a sequence as set forth in SEQ ID NO:15, or a subsequence thereof.

69. The transgenic seed of claim 62, wherein the seed is a corn seed, a wheat kernel, an oilseed, a rapeseed, a soybean seed, a palm kernel, a sunflower seed, a sesame seed, a rice, a barley, a peanut or a tobacco plant seed.

70. An antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to

- (i) a sequence as set forth in SEQ ID NO:1, or a subsequence thereof,  
a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:5 over a region of at least about 100 residues,  
a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:7 over a region of at least about 100 residues,  
a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:11 over a region of at least about 100 residues,  
a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:13 over a region of at least about 100 residues, or  
a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:15 over a region of at least about 100 residues,

wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection; or,

(ii) a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:5, or a subsequence thereof, a sequence as set forth in SEQ ID NO:7, or a subsequence thereof, a sequence as set forth in SEQ ID NO:11, or a subsequence thereof, a sequence as set forth in SEQ ID NO:13, or a subsequence thereof, a sequence as set forth in SEQ ID NO:15, or a subsequence thereof.

71. The antisense oligonucleotide of claim 70, wherein the antisense oligonucleotide is between about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, or about 60 to 100 bases in length.

5 72. A method of inhibiting the translation of an amylase message in a cell comprising administering to the cell or expressing in the cell an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a nucleic acid comprising

(i) a nucleic acid sequence as set forth in SEQ ID NO:1,

10 a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:5 over a region of at least about 100 residues,

a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:7 over a region of at least about 100 residues,

15 a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:11 over a region of at least about 100 residues,

a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:13 over a region of at least about 100 residues, or

a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:15 over a region of at least about 100 residues,

20 wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection; or,

(ii) a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:5, or a subsequence thereof, a sequence as set forth in SEQ ID NO:7, or a subsequence thereof, a sequence as set forth in SEQ ID NO:11, or a subsequence thereof, a sequence as set forth in SEQ ID NO:13, or a subsequence thereof, a sequence as set forth in SEQ ID NO:15, or a subsequence thereof.

73. An isolated or recombinant polypeptide comprising

30 (a) a polypeptide comprising

an amino acid sequence as set forth in SEQ ID NO:2,

an amino acid sequence having at least 90% identity to SEQ ID NO:6 over a region of at least about 100 residues,

an amino acid sequence having at least 60% identity to SEQ ID NO:8 over a region of at least about 100 residues,

an amino acid sequence having at least 50% identity to SEQ ID NO:12 over a region of at least about 100 residues,

5 an amino acid sequence having at least 70% identity to SEQ ID NO:14 over a region of at least about 100 residues, or

an amino acid sequence having at least 80% identity to SEQ ID NO:16 over a region of at least about 100 residues, or

(b) a polypeptide encoded by a nucleic acid comprising

10 (i) a nucleic acid sequence as set forth in SEQ ID NO:1,

a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:5 over a region of at least about 100 residues,

a nucleic acid sequence having at least 60% sequence identity to SEQ ID NO:7 over a region of at least about 100 residues,

15 a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:11 over a region of at least about 100 residues,

a nucleic acid sequence having at least 70% sequence identity to SEQ ID NO:13 over a region of at least about 100 residues, or

20 a nucleic acid sequence having at least 80% sequence identity to SEQ ID NO:15 over a region of at least about 100 residues,

wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection; or,

25 (ii) a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:5, or a subsequence thereof, a sequence as set forth in SEQ ID NO:7, or a subsequence thereof, a sequence as set forth in SEQ ID NO:11, or a subsequence thereof, a sequence as set forth in SEQ ID NO:13, or a subsequence thereof, a sequence as set forth in SEQ ID NO:15, or a subsequence thereof.

30 74. The isolated or recombinant polypeptide of claim 73, wherein the polypeptide has an amylase activity.

75. The isolated or recombinant polypeptide of claim 73, wherein the polypeptide comprises

an amino acid sequence having at least 90% identity to SEQ ID NO:6 over a region of at least about 200 residues,

an amino acid sequence having at least 60% identity to SEQ ID NO:8 over a region of at least about 200 residues,

5 an amino acid sequence having at least 50% identity to SEQ ID NO:12 over a region of at least about 200 residues,

an amino acid sequence having at least 70% identity to SEQ ID NO:14 over a region of at least about 200 residues, or

10 an amino acid sequence having at least 80% identity to SEQ ID NO:16 over a region of at least about 200 residues.

76. The isolated or recombinant polypeptide of claim 75, wherein the polypeptide comprises

15 an amino acid sequence having at least 90% identity to SEQ ID NO:6 over a region of at least about 300 residues,

an amino acid sequence having at least 60% identity to SEQ ID NO:8 over a region of at least about 300 residues,

an amino acid sequence having at least 50% identity to SEQ ID NO:12 over a region of at least about 300 residues,

20 an amino acid sequence having at least 70% identity to SEQ ID NO:14 over a region of at least about 300 residues, or

an amino acid sequence having at least 80% identity to SEQ ID NO:16 over a region of at least about 300 residues.

25 77. The isolated or recombinant polypeptide of claim 76, wherein the polypeptide comprises

an amino acid sequence having at least 90% identity to SEQ ID NO:6 over a region of at least about 400 residues,

30 an amino acid sequence having at least 60% identity to SEQ ID NO:8 over a region of at least about 400 residues,

an amino acid sequence having at least 50% identity to SEQ ID NO:12 over a region of at least about 400 residues,

an amino acid sequence having at least 70% identity to SEQ ID NO:14 over a region of at least about 400 residues, or

an amino acid sequence having at least 80% identity to SEQ ID NO:16 over a region of at least about 400 residues.

78. The isolated or recombinant polypeptide of claim 77, wherein the polypeptide comprises

an amino acid sequence having at least 90% identity to SEQ ID NO:6 over a region of at least about 500 residues,

an amino acid sequence having at least 60% identity to SEQ ID NO:8 over a region of at least about 500 residues,

an amino acid sequence having at least 50% identity to SEQ ID NO:12 over a region of at least about 500 residues,

an amino acid sequence having at least 70% identity to SEQ ID NO:14 over a region of at least about 500 residues, or

an amino acid sequence having at least 80% identity to SEQ ID NO:16 over a region of at least about 500 residues.

79. The isolated or recombinant polypeptide of claim 78, wherein the polypeptide comprises

an amino acid sequence having at least 95% identity to SEQ ID NO:6 over a region of at least about 100 residues,

an amino acid sequence having at least 70% identity to SEQ ID NO:8 over a region of at least about 100 residues,

an amino acid sequence having at least 60% identity to SEQ ID NO:12 over a region of at least about 100 residues,

an amino acid sequence having at least 80% identity to SEQ ID NO:14 over a region of at least about 100 residues, or

an amino acid sequence having at least 90% identity to SEQ ID NO:16 over a region of at least about 100 residues.

80. The isolated or recombinant polypeptide of claim 79, wherein the polypeptide comprises

an amino acid sequence having at least 98% identity to SEQ ID NO:6 over a region of at least about 100 residues,

an amino acid sequence having at least 80% identity to SEQ ID NO:8 over a region of at least about 100 residues,

an amino acid sequence having at least 70% identity to SEQ ID NO:12 over a region of at least about 100 residues,

5 an amino acid sequence having at least 90% identity to SEQ ID NO:14 over a region of at least about 100 residues, or

an amino acid sequence having at least 95% identity to SEQ ID NO:16 over a region of at least about 100 residues.

81. The isolated or recombinant polypeptide of claim 80, wherein the polypeptide comprises

an amino acid sequence having at least 99% identity to SEQ ID NO:6 over a region of at least about 100 residues,

15 an amino acid sequence having at least 90% identity to SEQ ID NO:8 over a region of at least about 100 residues,

an amino acid sequence having at least 80% identity to SEQ ID NO:12 over a region of at least about 100 residues,

an amino acid sequence having at least 95% identity to SEQ ID NO:14 over a region of at least about 100 residues, or

20 an amino acid sequence having at least 98% identity to SEQ ID NO:16 over a region of at least about 100 residues.

82. The isolated or recombinant polypeptide of claim 81, wherein the polypeptide comprises

25 an amino acid sequence having at least 95% identity to SEQ ID NO:8 over a region of at least about 100 residues,

an amino acid sequence having at least 90% identity to SEQ ID NO:12 over a region of at least about 100 residues,

30 an amino acid sequence having at least 98% identity to SEQ ID NO:14 over a region of at least about 100 residues, or

an amino acid sequence having at least 99% identity to SEQ ID NO:16 over a region of at least about 100 residues.

83. The isolated or recombinant polypeptide of claim 82, wherein the polypeptide comprises

an amino acid sequence having at least 98% identity to SEQ ID NO:8 over a region of at least about 100 residues,

an amino acid sequence having at least 95% identity to SEQ ID NO:12 over a region of at least about 100 residues, or

an amino acid sequence having at least 99% identity to SEQ ID NO:14 over a region of at least about 100 residues.

84. The isolated or recombinant polypeptide of claim 83, wherein the polypeptide comprises

an amino acid sequence having at least 99% identity to SEQ ID NO:8 over a region of at least about 100 residues,

an amino acid sequence having at least 98% identity to SEQ ID NO:12 over a region of at least about 100 residues.

85. The isolated or recombinant polypeptide of claim 84, wherein the polypeptide comprises an amino acid sequence having at least 99% identity to SEQ ID NO:12 over a region of at least about 100 residues.

86. The isolated or recombinant polypeptide of claim 85, wherein the polypeptide comprises an amino acid sequence as set forth in SEQ ID NO:2, an amino acid sequence as set forth in SEQ ID NO:6, an amino acid sequence as set forth in SEQ ID NO:8, an amino acid sequence as set forth in SEQ ID NO:12, an amino acid sequence as set forth in SEQ ID NO:14, or an amino acid sequence as set forth in SEQ ID NO:16

87. The isolated or recombinant polypeptide of claim 74, wherein the amylase activity comprises hydrolyzing glucosidic bonds.

88. The isolated or recombinant polypeptide of claim 87, wherein the amylase activity comprises a glucoamylase activity.

89. The isolated or recombinant polypeptide of claim 87, wherein the amylase activity comprises a 1,4- $\alpha$ -D-glucan glucohydrolase activity.



90. The isolated or recombinant polypeptide of claim 87, wherein the amylase activity comprises an  $\alpha$ -amylase activity.

5 91. The isolated or recombinant polypeptide of claim 87, wherein the amylase activity comprises an exoamylase activity.

92. The isolated or recombinant polypeptide of claim 87, wherein the amylase activity comprises a  $\beta$ -amylase activity.

10 93. The isolated or recombinant polypeptide of claim 87, wherein the glucosidic bonds comprise an  $\alpha$ -1,4-glucosidic bond.

15 94. The isolated or recombinant polypeptide of claim 87, wherein the glucosidic bonds comprise an  $\alpha$ -1,6-glucosidic bond.

95. The isolated or recombinant polypeptide of claim 87, wherein the amylase activity comprises hydrolyzing glucosidic bonds in a starch.

20 96. The isolated or recombinant polypeptide of claim 95, wherein the amylase activity further comprises hydrolyzing glucosidic bonds in the starch to produce maltodextrines.

25 97. The isolated or recombinant polypeptide of claim 87, wherein the amylase activity comprises cleaving a maltose or a D-glucose unit from non-reducing end of the starch.

98. The isolated or recombinant polypeptide of claim 74, wherein the amylase activity is thermostable.

30 99. The isolated or recombinant polypeptide of claim 98, wherein the polypeptide retains an amylase activity under conditions comprising a temperature range of between about 37°C to about 95°C.

100. The isolated or recombinant polypeptide of claim 99, wherein the polypeptide retains an amylase activity under conditions comprising a temperature range of between about 55°C to about 85°C.

5 101. The isolated or recombinant polypeptide of claim 100, wherein the polypeptide retains an amylase activity under conditions comprising a temperature range of between about 70°C to about 95°C.

10 102. The isolated or recombinant polypeptide of claim 101, wherein the polypeptide retains an amylase activity under conditions comprising a temperature range of between about 90°C to about 95°C.

15 103. The isolated or recombinant polypeptide of claim 74, wherein the amylase activity is thermotolerant.

104. The isolated or recombinant polypeptide of claim 103, wherein the polypeptide retains an amylase activity after exposure to a temperature in the range from greater than 37°C to about 95°C.

20 105. The isolated or recombinant polypeptide of claim 104, wherein the polypeptide retains an amylase activity after exposure to a temperature in the range from greater than 55°C to about 85°C.

25 106. The isolated or recombinant polypeptide of claim 104, wherein the polypeptide retains an amylase activity after exposure to a temperature in the range from greater than 90°C to about 95°C.

107. An isolated or recombinant polypeptide comprising the polypeptide as set forth in claim 73 and lacking a signal sequence.

30 108. The isolated or recombinant polypeptide of claim 74, wherein the amylase activity comprises a specific activity at about 37°C in the range from about 100 to about 1000 units per milligram of protein.

109. The isolated or recombinant polypeptide of claim 108, wherein the amylase activity comprises a specific activity from about 500 to about 750 units per milligram of protein.

5 110. The isolated or recombinant polypeptide of claim 74, wherein the amylase activity comprises a specific activity at 37°C in the range from about 500 to about 1200 units per milligram of protein.

10 111. The isolated or recombinant polypeptide of claim 110, wherein the amylase activity comprises a specific activity at 37°C in the range from about 750 to about 1000 units per milligram of protein.

15 112. The isolated or recombinant polypeptide of claim 103, wherein the thermotolerance comprises retention of at least half of the specific activity of the amylase at 37°C after being heated to an elevated temperature.

20 113. The isolated or recombinant polypeptide of claim 112, wherein the thermotolerance comprises retention of specific activity at 37°C in the range from about 500 to about 1200 units per milligram of protein after being heated to an elevated temperature.

114. The isolated or recombinant polypeptide of claim 73, wherein the polypeptide comprises at least one glycosylation site.

25 115. The isolated or recombinant polypeptide of claim 114, wherein glycosylation is an N-linked glycosylation.

116. The isolated or recombinant polypeptide of claim 114, wherein amylase is glycosylated after being expressed in a *P. pastoris* or a *S. pombe*.

30 117. The isolated or recombinant polypeptide of claim 74, wherein the polypeptide retains an amylase activity under conditions comprising about pH 5.

118. The isolated or recombinant polypeptide of claim 117, wherein the polypeptide retains an amylase activity under conditions comprising about pH 4.5.

119. The isolated or recombinant polypeptide of claim 74, wherein the polypeptide retains an amylase activity under conditions comprising about pH 8.0.

5 120. The isolated or recombinant polypeptide of claim 119 wherein the polypeptide retains an amylase activity under conditions comprising about pH 8.5.

121. The isolated or recombinant polypeptide of claim 120, wherein the polypeptide retains an amylase activity under conditions comprising about pH 9.

10 122. The isolated or recombinant polypeptide of claim 121, wherein the polypeptide retains an amylase activity under conditions comprising about pH 9.5.

15 123. The isolated or recombinant polypeptide of claim 122, wherein the polypeptide retains an amylase activity under conditions comprising about pH 10.

124. The isolated or recombinant polypeptide of claim 123, wherein the polypeptide retains an amylase activity under conditions comprising about pH 10.5.

20 125. A protein preparation comprising a polypeptide as set forth in claim 73, wherein the protein preparation comprises a liquid, a solid or a gel.

126. A heterodimer comprising a polypeptide as set forth in claim 73 and a second domain.

25 127. The heterodimer of claim 126, wherein the second domain is a polypeptide and the heterodimer is a fusion protein.

30 128. The heterodimer of claim 126, wherein the second domain is an epitope.

129. The heterodimer of claim 126, wherein the second domain is a tag.

130. A homodimer comprising a polypeptide as set forth in claim 73.

131. An immobilized polypeptide having an amylase activity, wherein the polypeptide comprises a sequence as set forth in claim 73 or claim 126.

5 132. The immobilized polypeptide of claim 131, wherein the polypeptide is immobilized on a cell, a metal, a resin, a polymer, a ceramic, a glass, a microelectrode, a partic particle, a bead, a gel, a plate, an array or a capillary tube.

133. An array comprising an immobilized polypeptide as set forth in claim 73 or claim 126.

134. An array comprising an immobilized nucleic acid as set forth in claim 1 or claim 41.

15 135. An isolated or recombinant antibody that specifically binds to a polypeptide as set forth in claim 73 or to a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41.

20 136. The isolated or recombinant antibody of claim 135, wherein the antibody is a monoclonal or a polyclonal antibody.

137. A hybridoma comprising an antibody that specifically binds to a polypeptide as set forth in claim 73 or to a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41.

25 138. A food supplement for an animal comprising:  
a polypeptide as set forth in claim 73;  
a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41;  
a polypeptide comprising an amino acid sequence having at least 90%  
30 identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:10 over a region of at least about 100 residues; or

a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:9 or a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as

set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:3, or a subsequence thereof, a sequence as set forth in SEQ ID NO:9, or a subsequence thereof.

5                   139.   The food supplement of claim 138, wherein the polypeptide is glycosylated.

                  140.   An edible enzyme delivery matrix comprising:  
                  a polypeptide as set forth in claim 73;  
10               a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41;  
                  a polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:10 over a region of at least about 100 residues; or

                  a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence  
15               having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:9 or a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:3, or a subsequence thereof, a sequence as set forth in SEQ ID NO:9, or a subsequence thereof.

20                   141.   The edible enzyme delivery matrix of claim 140, wherein the delivery matrix comprises a pellet.

                  142.   The edible enzyme delivery matrix of claim 140, wherein the  
25               polypeptide is glycosylated.

                  143.   The edible enzyme delivery matrix of claim 140, wherein the amylase activity is thermotolerant.

30                   144.   The edible enzyme delivery matrix of claim 140, wherein the amylase activity is thermostable.

                  145.   A method of isolating or identifying a polypeptide with an amylase activity comprising the steps of:

(a) providing an antibody as set forth in claim 135, or an antibody that specifically binds to a polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:10 over a region of at least about 100 residues; or a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:9 or a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:3, or a subsequence thereof, a sequence as set forth in SEQ ID NO:9, or a subsequence thereof;

(b) providing a sample comprising polypeptides; and

(c) contacting the sample of step (b) with the antibody of step (a) under conditions wherein the antibody can specifically bind to the polypeptide, thereby isolating or identifying a polypeptide having an amylase activity.

146. A method of making an anti-amylase antibody comprising administering to a non-human animal a nucleic acid as set forth in claim 1 or claim 41, a polypeptide as set forth in claim 73, a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41; a polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:10 over a region of at least about 100 residues; or a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:9 or a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:3, or a subsequence thereof, a sequence as set forth in SEQ ID NO:9, or a subsequence thereof in an amount sufficient to generate a humoral immune response, thereby making an anti-amylase antibody.

147. A method of producing a recombinant polypeptide comprising the steps of:

(a) providing a nucleic acid operably linked to a promoter, wherein the nucleic acid comprises a sequence as set forth in claim 1 or claim 41; and

(b) expressing the nucleic acid of step (a) under conditions that allow expression of the polypeptide, thereby producing a recombinant polypeptide.

148. The method of claim 147, further comprising transforming a host cell with the nucleic acid of step (a) followed by expressing the nucleic acid of step (a), thereby producing a recombinant polypeptide in a transformed cell.

5 149. A method for identifying a polypeptide having an amylase activity comprising the following steps:

(a) providing a polypeptide as set forth in claim 73; or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41;

(b) providing an amylase substrate; and

10 (c) contacting the polypeptide with the substrate of step (b) and detecting a decrease in the amount of substrate or an increase in the amount of a reaction product, wherein a decrease in the amount of the substrate or an increase in the amount of the reaction product detects a polypeptide having an amylase activity.

15 150. The method of claim 149 wherein the substrate is a starch.

151. A method for identifying an amylase substrate comprising the following steps:

20 (a) providing a polypeptide as set forth in claim 73; or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41;

(b) providing a test substrate; and

25 (c) contacting the polypeptide of step (a) with the test substrate of step (b) and detecting a decrease in the amount of substrate or an increase in the amount of reaction product, wherein a decrease in the amount of the substrate or an increase in the amount of a reaction product identifies the test substrate as an amylase substrate.

152. A method of determining whether a test compound specifically binds to a polypeptide comprising the following steps:

30 (a) expressing a nucleic acid or a vector comprising the nucleic acid under conditions permissive for translation of the nucleic acid to a polypeptide, wherein the nucleic acid has a sequence as set forth in claim 1 or claim 41, or, providing a polypeptide as set forth in claim 73;

(b) providing a test compound;

(c) contacting the polypeptide with the test compound; and



(d) determining whether the test compound of step (b) specifically binds to the polypeptide.

153. A method for identifying a modulator of an amylase activity comprising the following steps:

(a) providing a polypeptide as set forth in claim 74 or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41;

(b) providing a test compound;

(c) contacting the polypeptide of step (a) with the test compound of step (b) and measuring an activity of the amylase, wherein a change in the amylase activity measured in the presence of the test compound compared to the activity in the absence of the test compound provides a determination that the test compound modulates the amylase activity.

154. The method of claim 153, wherein the amylase activity is measured by providing an amylase substrate and detecting a decrease in the amount of the substrate or an increase in the amount of a reaction product, or, an increase in the amount of the substrate or a decrease in the amount of a reaction product.

155. The method of claim 154, wherein a decrease in the amount of the substrate or an increase in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an activator of amylase activity.

156. The method of claim 154, wherein an increase in the amount of the substrate or a decrease in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an inhibitor of amylase activity.

157. A computer system comprising a processor and a data storage device wherein said data storage device has stored thereon a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises sequence as set forth in claim 73, a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41.

158. The computer system of claim 157, further comprising a sequence comparison algorithm and a data storage device having at least one reference sequence stored thereon.

5 159. The computer system of claim 158, wherein the sequence comparison algorithm comprises a computer program that indicates polymorphisms.

160. The computer system of claim 157, further comprising an identifier that identifies one or more features in said sequence.

10 161. A computer readable medium having stored thereon a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises a polypeptide as set forth in claim 73; a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41.

15 162. A method for identifying a feature in a sequence comprising the steps of:

(a) reading the sequence using a computer program which identifies one or more features in a sequence, wherein the sequence comprises a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises a polypeptide as set forth in claim 73; a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41; and

(b) identifying one or more features in the sequence with the computer program.

25 163. A method for comparing a first sequence to a second sequence comprising the steps of:

(a) reading the first sequence and the second sequence through use of a computer program which compares sequences, wherein the first sequence comprises a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises a polypeptide as set forth in claim 73 or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41; and

(b) determining differences between the first sequence and the second sequence with the computer program.

164. The method of claim 163, wherein the step of determining differences between the first sequence and the second sequence further comprises the step of identifying polymorphisms.

5

165. The method of claim 163, further comprising an identifier that identifies one or more features in a sequence.

166. The method of claim 163, comprising reading the first sequence with a computer program and identifying one or more features in the sequence.

167. A method for isolating or recovering a nucleic acid encoding a polypeptide with an amylase activity from an environmental sample comprising the steps of:

15

(a) providing an amplification primer sequence pair for amplifying a nucleic acid encoding a polypeptide with an amylase activity, wherein the primer pair is capable of amplifying SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, or a subsequence thereof;

20

(b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to the amplification primer pair; and,

25

(c) combining the nucleic acid of step (b) with the amplification primer pair of step (a) and amplifying nucleic acid from the environmental sample, thereby isolating or recovering a nucleic acid encoding a polypeptide with an amylase activity from an environmental sample.

30

168. The method of claim 167, wherein each member of the amplification primer sequence pair comprises an oligonucleotide comprising at least about 10 to 50 consecutive bases of a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, or a subsequence thereof.

109. A method for isolating or recovering a nucleic acid encoding a polypeptide with an amylase activity from an environmental sample comprising the steps of:

5 (a) providing a polynucleotide probe comprising a sequence as set forth in claim 1 or claim 41 or a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:9, or a subsequence thereof;

(b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to a polynucleotide probe of step (a);

10 (c) combining the isolated nucleic acid or the treated environmental sample of step (b) with the polynucleotide probe of step (a); and

(d) isolating a nucleic acid that specifically hybridizes with the polynucleotide probe of step (a), thereby isolating or recovering a nucleic acid encoding a polypeptide with an amylase activity from an environmental sample.

15 170. The method of claim 167 or claim 169, wherein the environmental sample comprises a water sample, a liquid sample, a soil sample, an air sample or a biological sample.

20 171. The method of claim 170, wherein the biological sample is derived from a bacterial cell, a protozoan cell, an insect cell, a yeast cell, a plant cell, a fungal cell or a mammalian cell.

25 172. A method of generating a variant of a nucleic acid encoding a polypeptide with an amylase activity comprising the steps of:

(a) providing a template nucleic acid comprising a sequence as set forth in claim 1 or claim 41 or a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:9; and

30 (b) modifying, deleting or adding one or more nucleotides in the template sequence, or a combination thereof, to generate a variant of the template nucleic acid.

173. The method of claim 172, further comprising expressing the variant nucleic acid to generate a variant amylase polypeptide.

174. The method of claim 172, wherein the modifications, additions or deletions are introduced by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturated mutagenesis (GSSM), synthetic ligation reassembly (SLR) and a combination thereof.

175. The method of claim 172, wherein the modifications, additions or deletions are introduced by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof.

176. The method of claim 172, wherein the modifications, additions or deletions are introduced by error-prone PCR.

177. The method of claim 172, wherein the modifications, additions or deletions are introduced by shuffling.

178. The method of claim 172, wherein the modifications, additions or deletions are introduced by oligonucleotide-directed mutagenesis.

179. The method of claim 172, wherein the modifications, additions or deletions are introduced by assembly PCR.

180. The method of claim 172, wherein the modifications, additions or deletions are introduced by sexual PCR mutagenesis.

181. The method of claim 172, wherein the modifications, additions or deletions are introduced by in vivo mutagenesis.

182. The method of claim 172, wherein the modifications, additions or deletions are introduced by cassette mutagenesis.

183. The method of claim 172, wherein the modifications, additions or deletions are introduced by recursive ensemble mutagenesis.

184. The method of claim 172, wherein the modifications, additions or deletions are introduced by exponential ensemble mutagenesis.

185. The method of claim 172, wherein the modifications, additions or deletions are introduced by site-specific mutagenesis.

186. The method of claim 172, wherein the modifications, additions or deletions are introduced by gene reassembly.

187. The method of claim 172, wherein the modifications, additions or deletions are introduced by synthetic ligation reassembly (SLR).

188. The method of claim 172, wherein the modifications, additions or deletions are introduced by gene site saturated mutagenesis (GSSM).

189. The method of claim 172, wherein the method is iteratively repeated until an amylase having an altered or different activity or an altered or different stability from that of a polypeptide encoded by the template nucleic acid is produced.

190. The method of claim 189, wherein the variant amylase polypeptide is thermotolerant, and retains some activity after being exposed to an elevated temperature.

191. The method of claim 189, wherein the variant amylase polypeptide has increased glycosylation as compared to the amylase encoded by a template nucleic acid.

192. The method of claim 189, wherein the variant amylase polypeptide has an amylase activity under a high temperature, wherein the amylase encoded by the template nucleic acid is not active under the high temperature.

5 193. The method of claim 172, wherein the method is iteratively repeated until an amylase coding sequence having an altered codon usage from that of the template nucleic acid is produced.

10 194. The method of claim 172, wherein the method is iteratively repeated until an amylase gene having higher or lower level of message expression or stability from that of the template nucleic acid is produced.

15 195. A method for modifying codons in a nucleic acid encoding a polypeptide with an amylase activity to increase its expression in a host cell, the method comprising the following steps:

(a) providing a nucleic acid encoding a polypeptide with an amylase activity comprising a sequence as set forth in claim 1 or claim 41 or a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:9; and,

20 (b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

25 196. A method for modifying codons in a nucleic acid encoding an amylase polypeptide, the method comprising the following steps:

30 (a) providing a nucleic acid encoding a polypeptide with an amylase activity comprising a sequence as set forth in claim 1 or claim 41 or a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:9; and,

(b) identifying a codon in the nucleic acid of step (a) and replacing it with a different codon encoding the same amino acid as the replaced codon, thereby modifying codons in a nucleic acid encoding an amylase.

197. A method for modifying codons in a nucleic acid encoding an amylase polypeptide to increase its expression in a host cell, the method comprising the following steps:

(a) providing a nucleic acid encoding an amylase polypeptide comprising a sequence as set forth in claim 1 or claim 41 or a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:9; and,

(b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

198. A method for modifying a codon in a nucleic acid encoding a polypeptide having an amylase activity to decrease its expression in a host cell, the method comprising the following steps:

(a) providing a nucleic acid encoding an amylase polypeptide comprising a sequence as set forth in claim 1 or claim 41 or a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:9; and

(b) identifying at least one preferred codon in the nucleic acid of step (a) and replacing it with a non-preferred or less preferred codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in a host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to decrease its expression in a host cell.

199. The method of claim 197 or 198, wherein the host cell is a bacterial cell, a fungal cell, an insect cell, a yeast cell, a plant cell or a mammalian cell.

200. A method for producing a library of nucleic acids encoding a plurality of modified amylase active sites or substrate binding sites, wherein the modified active sites or substrate binding sites are derived from a first nucleic acid comprising a sequence encoding a first active site or a first substrate binding site the method comprising the following steps:



(a) providing a first nucleic acid encoding a first active site or first substrate binding site, wherein the first nucleic acid sequence comprises a sequence that hybridizes under stringent conditions to a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, or a subsequence thereof, and the nucleic acid encodes an amylase active site or an amylase substrate binding site;

(b) providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and,

(c) using the set of mutagenic oligonucleotides to generate a set of active site-encoding or substrate binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized, thereby producing a library of nucleic acids encoding a plurality of modified amylase active sites or substrate binding sites.

201. The method of claim 200, comprising mutagenizing the first nucleic acid of step (a) by a method comprising an optimized directed evolution system.

202. The method of claim 200, comprising mutagenizing the first nucleic acid of step (a) by a method comprising gene site-saturation mutagenesis (GSSM).

203. The method of claim 200, comprising mutagenizing the first nucleic acid of step (a) by a method comprising a synthetic ligation reassembly (SLR).

204. The method of claim 200, further comprising mutagenizing the first nucleic acid of step (a) or variants by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturated mutagenesis (GSSM), synthetic ligation reassembly (SLR) and a combination thereof.

205. The method of claim 200, further comprising mutagenizing the first nucleic acid of step (a) or variants by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing

template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof.

206. A method for making a small molecule comprising the following steps:

(a) providing a plurality of biosynthetic enzymes capable of synthesizing or modifying a small molecule, wherein one of the enzymes comprises an amylase enzyme encoded by a nucleic acid comprising a sequence as set forth in claim 1 or claim 41 or a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:9;

(b) providing a substrate for at least one of the enzymes of step (a); and

(c) reacting the substrate of step (b) with the enzymes under conditions that facilitate a plurality of biocatalytic reactions to generate a small molecule by a series of biocatalytic reactions.

207. A method for modifying a small molecule comprising the following steps:

(a) providing an amylase enzyme, wherein the enzyme comprises a polypeptide as set forth in claim 73;  
a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41;  
a polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:10 over a region of at least about 100 residues; or

a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:9 or a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:3, or a subsequence thereof, a sequence as set forth in SEQ ID NO:9, or a subsequence thereof;

(b) providing a small molecule; and

(c) reacting the enzyme of step (a) with the small molecule of step (b) under conditions that facilitate an enzymatic reaction catalyzed by the amylase enzyme, thereby modifying a small molecule by an amylase enzymatic reaction.

5           208.    The method of claim 207, comprising a plurality of small molecule substrates for the enzyme of step (a), thereby generating a library of modified small molecules produced by at least one enzymatic reaction catalyzed by the amylase enzyme.

10           209.    The method of claim 207, further comprising a plurality of additional enzymes under conditions that facilitate a plurality of biocatalytic reactions by the enzymes to form a library of modified small molecules produced by the plurality of enzymatic reactions.

15           210.    The method of claim 207, further comprising the step of testing the library to determine if a particular modified small molecule which exhibits a desired activity is present within the library.

20           211.    The method of claim 210, wherein the step of testing the library further comprises the steps of systematically eliminating all but one of the biocatalytic reactions used to produce a portion of the plurality of the modified small molecules within the library by testing the portion of the modified small molecule for the presence or absence of the particular modified small molecule with a desired activity, and identifying at least one specific biocatalytic reaction that produces the particular modified small molecule of desired activity.

25           212.    A method for determining a functional fragment of an amylase enzyme comprising the steps of:

            (a) providing an amylase enzyme, wherein the enzyme comprises  
            a polypeptide as set forth in claim 73;  
30           a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41;  
            a polypeptide comprising an amino acid sequence having at least 90%  
identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:10 over a region of at least about 100  
residues; or

a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:9 or a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:3, or a subsequence thereof, a sequence as set forth in SEQ ID NO:9, or a subsequence thereof; and

(b) deleting a plurality of amino acid residues from the sequence of step (a) and testing the remaining subsequence for an amylase activity, thereby determining a functional fragment of an amylase enzyme.

213. The method of claim 212, wherein the amylase activity is measured by providing an amylase substrate and detecting a decrease in the amount of the substrate or an increase in the amount of a reaction product.

214. A method for whole cell engineering of new or modified phenotypes by using real-time metabolic flux analysis, the method comprising the following steps:

(a) making a modified cell by modifying the genetic composition of a cell, wherein the genetic composition is modified by addition to the cell of a nucleic acid comprising a sequence as set forth in claim 1 or claim 41 or a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:9;

(b) culturing the modified cell to generate a plurality of modified cells;

(c) measuring at least one metabolic parameter of the cell by monitoring the cell culture of step (b) in real time; and,

(d) analyzing the data of step (c) to determine if the measured parameter differs from a comparable measurement in an unmodified cell under similar conditions, thereby identifying an engineered phenotype in the cell using real-time metabolic flux analysis.

215. The method of claim 214, wherein the genetic composition of the cell is modified by a method comprising deletion of a sequence or modification of a sequence in the cell, or, knocking out the expression of a gene.

216. The method of claim 214, further comprising selecting a cell comprising a newly engineered phenotype.

217. The method of claim 216, further comprising culturing the selected cell, thereby generating a new cell strain comprising a newly engineered phenotype.

218. A method for hydrolyzing a starch comprising the following steps:

(a) providing a polypeptide having an amylase activity, wherein the polypeptide comprises

a polypeptide as set forth in claim 73;

a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41;

a polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:10 over a region of at least about 100 residues; or

a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:9 or a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:3, or a subsequence thereof, a sequence as set forth in SEQ ID NO:9, or a subsequence thereof;

(b) providing a composition comprising a starch; and

(c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the polypeptide hydrolyzes the starch.

219. The method as set forth in claim 218, wherein the composition comprises an  $\alpha$ -1,4-glucosidic bond.

220. The method as set forth in claim 218, wherein the composition comprises an  $\alpha$ -1,6-glucosidic bond.

221. A method for liquefying or removing a starch from a composition comprising the following steps:

(a) providing a polypeptide having an amylase activity, wherein the polypeptide comprises

a polypeptide as set forth in claim 73;

a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41;

a polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:10 over a region of at least about 100 residues; or

a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:9 or a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:3, or a subsequence thereof, a sequence as set forth in SEQ ID NO:9, or a subsequence thereof;

(b) providing a composition comprising a starch; and

(c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the polypeptide removes or liquefies the starch.

222. A method of increasing thermotolerance or thermostability of an amylase polypeptide, the method comprising glycosylating an amylase polypeptide, wherein the polypeptide comprises at least thirty contiguous amino acids of a polypeptide as set forth in claim 73; a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41; a polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:10 over a region of at least about 100 residues; or a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:9 or a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:3, or a subsequence thereof, a sequence as set forth in SEQ ID NO:9, or a subsequence thereof, thereby increasing the thermotolerance or thermostability of the amylase polypeptide.

223. The method of claim 222, wherein the amylase specific activity is thermostable or thermotolerant at a temperature in the range from greater than about 37°C to about 95°C.

224. A method for overexpressing a recombinant amylase polypeptide in a cell comprising expressing a vector comprising a nucleic acid comprising a nucleic acid sequence at least 50% sequence identity to the nucleic acid of claim 1 or claim 41 or a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, or SEQ

ID NO:9 over a region of at least about 100 residues, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection, wherein overexpression is effected by use of a high activity promoter, a dicistronic vector or by gene amplification of the vector.

5

225. A detergent composition comprising a polypeptide as set forth in claim 73; a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41; a polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:10 over a region of at least about 100 residues; or a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:9 or a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:3, or a subsequence thereof, a sequence as set forth in SEQ ID NO:9, or a subsequence thereof, wherein the polypeptide comprises an amylase activity.

15

226. The detergent composition of claim 225, wherein the amylase is a nonsurface-active amylase.

20

227. The detergent composition of claim 225, wherein the amylase is a surface-active amylase.

25

228. A method for washing an object comprising the following steps:  
(a) providing a composition comprising a polypeptide having an amylase activity, wherein the polypeptide comprises:

30

a polypeptide as set forth in claim 73;  
a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41;  
a polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:10 over a region of at least about 100 residues; or

a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:9 or a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth

in SEQ ID NO:3, or a subsequence thereof, a sequence as set forth in SEQ ID NO:9, or a subsequence thereof;

(b) providing an object; and

(c) contacting the polypeptide of step (a) and the object of step (b) under conditions wherein the composition can wash the object.

229. A method for hydrolyzing a starch in a feed or a food prior to consumption by an animal comprising the following steps:

(a) obtaining a feed material comprising a starch, wherein the starch can be hydrolyzed by a polypeptide having an amylase activity, wherein the polypeptide comprises:

a polypeptide as set forth in claim 73;

a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41;

a polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:10 over a region of at least about 100 residues; or

a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:9 or a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:3, or a subsequence thereof, a sequence as set forth in SEQ ID NO:9, or a subsequence thereof; and

(b) adding the polypeptide of step (a) to the feed or food material in an amount sufficient for a sufficient time period to cause hydrolysis of the starch and formation of a treated food or feed, thereby hydrolyzing the starch in the food or the feed prior to consumption by the animal.

230. The method as set forth in claim 229, wherein the food or feed comprises rice, corn, barley, wheat, legumes, or potato.

231. A method for textile desizing comprising the following steps:

(a) providing a polypeptide having an amylase activity, wherein the polypeptide comprises

a polypeptide as set forth in claim 73;



a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41;

a polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:10 over a region of at least about 100 residues; or

5 a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:9 or a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:3, or a subsequence thereof, a sequence as set forth in SEQ ID NO:9, or a subsequence  
10 thereof;

(b) providing a fabric; and

(c) contacting the polypeptide of step (a) and the fabric of step (b) under conditions wherein the amylase can desize the fabric.

15 232. A method for deinking of paper or fibers comprising the following steps:

(a) providing a polypeptide having an amylase activity, wherein the polypeptide comprises:

a polypeptide as set forth in claim 73;

20 a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41;

a polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:10 over a region of at least about 100 residues; or

a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence  
25 having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:9 or a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:3, or a subsequence thereof, a sequence as set forth in SEQ ID NO:9, or a subsequence thereof;

30 (b) providing a composition comprising paper or fiber; and

(c) contacting the polypeptide of step (a) and the composition of step (b) under conditions wherein the polypeptide can deink the paper or fiber.

233. A method for treatment of lignocellulosic fibers comprising the following steps:

(a) providing a polypeptide having an amylase activity, wherein the polypeptide comprises

5 a polypeptide as set forth in claim 73;  
a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41;  
a polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:10 over a region of at least about 100 residues; or

10 a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:9 or a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:3, or a subsequence thereof, a sequence as set forth in SEQ ID NO:9, or a subsequence  
15 thereof;

(b) providing a lignocellulosic fiber; and

(c) contacting the polypeptide of step (a) and the fiber of step (b) under conditions wherein the polypeptide can treat the fiber thereby improving the fiber properties.

20 234. A method for producing a high-maltose or a high-glucose syrup comprising the following steps:

(a) providing a polypeptide having an amylase activity, wherein the polypeptide comprises

25 a polypeptide as set forth in claim 73;  
a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41;  
a polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:10 over a region of at least about 100 residues; or

30 a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:9 or a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID

NO:3, or a subsequence thereof, a sequence as set forth in SEQ ID NO:9, or a subsequence thereof;

(b) providing a composition comprising a starch; and

(c) contacting the polypeptide of step (a) and the fabric of step (b) under conditions wherein the polypeptide of step (a) can hydrolyze the composition of step (b), thereby producing a high-maltose or a high-glucose syrup.

235. The method as set forth in claim 234, wherein the starch is from rice, corn, barley, wheat, legumes, potato, or sweet potato.

236. A method for improving the flow of the starch-containing production fluids comprising the following steps:

(a) providing a polypeptide having an amylase activity, wherein the polypeptide comprises

a polypeptide as set forth in claim 73;

a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41;

a polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:10 over a region of at least about 100 residues; or

a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:9 or a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:3, or a subsequence thereof, a sequence as set forth in SEQ ID NO:9, or a subsequence thereof;

(b) providing production fluid comprising a starch; and

(c) contacting the polypeptide of step (a) and the production fluid of step (b) under conditions wherein the amylase can hydrolyze the starch in the production fluid, thereby improving its flow by decreasing its density.

237. The method as set forth in claim 236, wherein the production fluid is from a subterranean formation.

238. An anti-staling composition comprising a polypeptide as set forth in claim 73; a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41; a polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:10 over a region of at least about 100 residues; or a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:9 or a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:3, or a subsequence thereof, a sequence as set forth in SEQ ID NO:9, or a subsequence thereof.

239. A method for preventing staling of a baked product comprising the following steps:

(a) providing a polypeptide comprising  
a polypeptide as set forth in claim 73;  
a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41;  
a polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:10 over a region of at least about 100 residues; or

a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:9 or a nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth in SEQ ID NO:3, or a subsequence thereof, a sequence as set forth in SEQ ID NO:9, or a subsequence thereof;

(b) providing a composition used for baking comprising a starch;  
(c) combining the polypeptide of step (a) with the composition of the step (b) under conditions wherein the polypeptide can hydrolyze the starch in the composition used for baking, thereby preventing staling of the baked product.

240. The method as set forth in claim 239, wherein the baked product is bread.

241. A method for using amylase in brewing or alcohol production comprising the following steps:

(a) providing a polypeptide comprising  
a polypeptide as set forth in claim 73;  
a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 41;  
a polypeptide comprising an amino acid sequence having at least 90%  
5 identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:10 over a region of at least about 100  
residues; or

a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence  
having at least 90% identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:9 or a  
nucleic acid that hybridizes under stringent conditions to a nucleic acid comprising a  
sequence as set forth in SEQ ID NO:1, or a subsequence thereof, a sequence as set forth  
in SEQ ID NO:3, or a subsequence thereof, a sequence as set forth in SEQ ID NO:9, or  
a subsequence thereof;

(b) providing a composition used for brewing or in alcohol production  
comprising a starch;

15 (c) combining the polypeptide of step (a) with the composition of the step  
(b) under conditions wherein the polypeptide can hydrolyze the starch in the composition  
used for brewing or alcohol production.

20 242. The method as set forth in claim 241, wherein the composition  
comprising a starch is a beer.

243. The expression cassette of claim 56, wherein the nucleic acid is  
operably linked to a plant promoter.

25 244. The expression cassette of claim 243 further comprising a plant  
expression vector.

245. The expression cassette of claim 244, wherein the plant expression  
vector comprises a plant virus.

30 246. The expression cassette of claim 243, wherein the plant promoter  
comprises a potato promoter.

247. The expression cassette of claim 243, wherein the plant promoter comprises a rice or corn promoter.

248. The expression cassette of claim 245, wherein the plant promoter comprises a wheat or barley promoter.

249. The expression cassette of claim 242, wherein the promoter comprises a promoter derived from T-DNA of *Agrobacterium tumefaciens*.

250. The expression cassette of claim 242, wherein the promoter is a constitutive promoter.

251. The expression cassette of claim 250, wherein the constitutive promoter is CaMV35S.

252. The expression cassette of claim 242, wherein the promoter is an inducible promoter.

253. The expression cassette of claim 242, wherein the promoter is a tissue-specific promoter.

254. The expression cassette of claim 253, wherein the tissue-specific promoter is a seed-specific, a leaf-specific, a root-specific, a stem-specific or an abscission-induced promoter.

255. The transformed cell of claim 61 or claim 62, wherein the cell is a plant cell.

256. The transformed cell of claim 255, wherein the plant cell is a potato, rice, corn, wheat, tobacco or barley cell.

257. A method of making a transgenic plant comprising the following steps:

(a) introducing a heterologous nucleic acid sequence into the cell, wherein the heterologous nucleic sequence comprises a sequence as set forth in claim 1 or claim 41, thereby producing a transformed plant cell;

(b) producing a transgenic plant from the transformed cell.

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258. The method as set forth in claim 257, wherein the step (a) further comprises introducing the heterologous nucleic acid sequence by electroporation or microinjection of plant cell protoplasts.

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259. The method as set forth in claim 257, wherein the step (a) further comprises introducing the heterologous nucleic acid sequence directly to plant tissue by DNA particle bombardment.

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260. The method as set forth in claim 257, wherein the step (a) further comprises introducing the heterologous nucleic acid sequence into the plant cell DNA using an *Agrobacterium tumefaciens* host.

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261. A method of expressing a heterologous nucleic acid sequence in a plant cell comprising the following steps:

(a) transforming the plant cell with a heterologous nucleic acid sequence operably linked to a promoter, wherein the heterologous nucleic sequence comprises a sequence as set forth in claim 1 or claim 41;

(b) growing the plant under conditions wherein the heterologous nucleic acids sequence is expressed in the plant cell.

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262. An amplification primer pair for amplifying a nucleic acid encoding a polypeptide having an amylase activity, wherein the primer pair is capable of amplifying a nucleic acid comprising a sequence as set forth in claim 1 or claim 24, or a subsequence thereof.

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263. The amplification primer pair of claim 262, wherein a member of the amplification primer sequence pair comprises an oligonucleotide comprising at least about 10 to 50 consecutive bases of the sequence, or, about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more consecutive bases of the sequence.

264. An amplification primer pair, wherein the primer pair comprises a first member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more residues of a sequence as set forth in claim 1 or claim 41, and a second member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more residues of the complementary strand of the first member.

265. An amylase-encoding nucleic acid generated by amplification of a polynucleotide using an amplification primer pair as set forth in claim 264.

266. The amylase-encoding nucleic acid of claim 265, wherein the amplification is by polymerase chain reaction (PCR).

267. The amylase-encoding nucleic acid of claim 265, wherein the nucleic acid generated by amplification of a gene library.

268. The amylase-encoding nucleic acid of claim 267, wherein the gene library is an environmental library.

269. An isolated or recombinant protease encoded by a protease-encoding nucleic acid as set forth in claim 265.

270. A method of amplifying a nucleic acid encoding a polypeptide having an amylase activity comprising amplification of a template nucleic acid with an amplification primer sequence pair capable of amplifying a nucleic acid sequence as set forth in claim 1 or claim 24, or a subsequence thereof.